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Liberation of vessel-adherent myeloperoxidase reflects plaque burden in patients with stable coronary artery disease

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ABSTRACT

Objective: Myeloperoxidase (MPO) has emerged as an important pathophysiological determinant of inflammatory vascular artery disease. It is appreciated that vessel immobilized, rather than circulating, MPO is critical for the progression of atherosclerotic lesions. The objective of this study was to investigate whether vessel-immobilized MPO is associated with the extent of coronary plaque burden.
Methods: MPO plasma levels were determined by ELISA before and after heparin-release of vessel-bound MPO, to study the relation between vascular MPO deposition and densitometrically assessed coronary plaque burden in 77 patients with stable coronary artery disease.
Results: Patients with a low increase in MPO plasma levels upon heparinization had a significantly smaller total plaque area and volume (12.1[IR:6.2–19.4]mm^2 vs. 19.8[IR:11.3–31.5]mm^2, $p < 0.01$; 27.8 [IR:12.3–44.8]mm^3 vs. 55.2[IR:24.2–87.5]mm^3, $p < 0.05$). Multivariable linear regression revealed that ΔMPO was independently associated with plaque area, and that ΔMPO increased with the number of affected vessels. Selective sampling confirmed the predominant role of coronary MPO deposition.
Conclusion: Our data demonstrate that heparin-induced mobilization of vessel-bound MPO is closely linked to coronary plaque burden and thus further corroborate the evidence for the intimate involvement of this enzyme in vascular pathophysiology, as well as the importance of inflammation in atherosclerosis.

Myeloperoxidase (MPO), a heme-enzyme abundantly expressed in polymorphonuclear neutrophils (PMN), is critically linked to all stages of arteriosclerotic disease [1]. MPO oxidizes vascular nitric oxide (NO, thereby deteriorating endothelial function) [2] and propagates arteriosclerotic plaque progression through its capacity to generate various reactive species [3]. Thus, MPO oxidizes low-density lipoprotein (LDL), thereby leading to enhanced foam cell formation [4]; and high-density lipoprotein (HDL), converting it to a dysfunctional and proatherogenic form [5]. MPO negatively influences eNOS expression and activity [6] and generates 3-nitrotyrosine, a hallmark of oxidative tissue injury [7]. In addition, MPO elicits cytokine-like properties which are independent of its catalytic activity, in that it activates PMN via CD11b/CD18 integrin [8]. Given its ability to activate matrix degrading metalloproteinases [9,10], MPO seems to be involved in plaque rupture [11].

After release from activated PMN, the cationic protein MPO accumulates in the subendothelial layer due to electrostatic interactions with the anionic glycocalyx and extracellular matrix [7,12]. Administration of heparin, which is strongly anionic, releases MPO from the vessel wall. In a previous study, our group was able to demonstrate that patients with stable coronary artery disease (CAD) had a higher degree of MPO liberation, reflecting increased systemic vascular deposition of MPO in CAD. Reversal of vascular immobilized MPO *in vivo* translated into improved flow-mediated dilation, a surrogate for endothelial NO-bioavailability [13].

The current study further extends these results and investigates whether vascular MPO deposition is indicative of the extent of

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Table 1
Patient baseline characteristics.

	ΔMPO < 533 pM ($n = 20$)	ΔMPO ≥ 533 pM ($n = 57$)	p
Age, y	68.1 ± 8.9	66.9 ± 10.3	0.66
Gender, female	4 (20.0)	12 (21.1)	0.59
BMI	26.2 ± 3.3	26.7 ± 3.7	0.64
Hypertension	19 (95.0)	52 (91.2)	0.59
Hypercholesterolemia	13 (65.0)	39 (68.4)	0.78
Diabetes	3 (15.0)	15 (26.3)	0.30
Smoking	6 (30.0)	12 (21.1)	0.42
NT-proBNP, pg/ml	152.6 ± 174.1	200.7 ± 234.5	0.43
hs-CRP, mg/dl	2.8 ± 3.7	4.3 ± 6.2	0.36
Leukocyte count, $10e^6$/ml	7.5 ± 2.3	7.2 ± 1.3	0.60
LDL, mg/dl	124.6 ± 46.3	110.1 ± 41.0	0.29
ACE-I, ARB	14 (70.0)	37 (64.9)	0.68
Betablocker	13 (65.0)	41 (71.9)	0.56
Statin	13 (65.0)	39 (68.4)	0.78

BMI: body mass index, hs-CRP: high sensitive C-reactive protein, LDL: low density lipoprotein, NT-pro BNP: N-terminal pro brain natriuretic peptide, ACE-I: angiotensin-converting enzyme inhibitor, ARB: angiotensin receptor blocker.
Values are given as mean ± standard deviation or frequency (percentage).

coronary artery disease in patients with CAD and seeks to assess if the coronary circulation is the predominant site of MPO deposition. Accordingly, MPO liberation upon heparinization was assessed in patients undergoing elective coronary angiography, allowing subsequent measurement of the extent of the angiographically detectable coronary plaque burden.

1. Methods

A detailed methods section is provided in the Online Supplement. Briefly, patients with stable CAD undergoing elective coronary angiography who showed at least one coronary stenosis with a narrowing of >25% of the lumen diameter were consecutively included. Venous blood samples were taken before and 15 min after heparinization (5000 U), where peak MPO plasma levels are observed after heparin administration [14]. Evaluation of plaque morphology was determined off-line using QUANTCOR QCA analysis software (Siemens, Erlangen, Germany) [15]. MPO plasma levels were assessed by CardioMPO Elisa (Cleveland HeartLab. Cleveland, OH, USA). To determine the main site of MPO release, blood samples were collected from the aorta, the coronary sinus and the femoral vein in a separate group of six patients at one, three and 5 min following heparin administration. To assess dose dependency of the heparin effect, collagen was incubated with MPO and increasing doses of heparin, and MPO activity was assessed. A detailed description of statistical analysis can be found in the Online Supplement.

2. Results

77 consecutive patients (mean age: 66.4 ± 12.6 y; 16% female) were included. 15 patients (19.5%) exhibited a 1-vessel, 33 (42.9%) a 2-vessel and 29 (37.7%) a 3-vessel disease (average stenosis: 58.9 ± 17.3%). The median MPO plasma levels were 449[IR:314–876]pM before heparin administration (MPO_{pre}), 1468[IR:1036–1915]pM after heparin administration (MPO_{post}). The mean increase in MPO plasma levels (ΔMPO) was 904.8 ± 698.2 pM. No correlation was observed between baseline MPO levels and ΔMPO (−0.176, $p = 0.125$). No significant differences in baseline characteristics were observed between patients within the lowest quartile of ΔMPO and those within the other quartiles (Table 1).

The total plaque burden expressed as area (PA_{total}) and volume (PV_{total}) was significantly higher in patients with high ΔMPO (19.8 [IR:11.3–31.5] vs. 12.1[IR:6.2–19.4]mm^2, $p < 0.01$; 55.2[IR:24.2–87.5] vs. 27.8[IR:12.3–44.8]mm^3, $p < 0.05$). Overall, ΔMPO showed a significant correlation with PA_{total} ($r = 0.24$; $p < 0.05$) and PV_{total} ($r = 0.29$; $p < 0.05$; Fig. 1). Similar differences between patients with high and low ΔMPO were observed for maximal plaque area (PA_{max}) and volume (10.3[IR:6.3–17.6] vs. 6.9[IR:4.9–9.2]mm^2, $p < 0.05$; 29.9[IR:16.0–51.0] vs. 18.1[IR:10.0–23.8]mm^3, $p < 0.01$; Fig. 1). Significant difference between quartiles was observed ($p < 0.05$), with levels of PA_{total} and PV_{total} increasing with ΔMPO quartiles ($r = 0.29$, $p < 0.05$; $r = 0.30$, $p < 0.01$). Post-hoc analysis revealed a significant difference between each of the three highest quartiles and the lowest quartile. However, no significant difference was observed between any of the highest three quartiles (Fig. 1). Separation of the single quartiles revealed significant differences between quartiles for PV_{max} ($p < 0.05$), with a trend towards differences between PA_{max} ($p = 0.11$).

Multivariable linear regression revealed that even after multivariate adjustment, ΔMPO remained independently associated with PA_{total}; with 12.4% of the variation in PA_{total} being explainable by ΔMPO (partial η^2: 0.124; $p < 0.013$).

No difference between patients with low and high MPO_{pre} or low and high MPO_{post} with regard to PA_{total}, PV_{total}, PA_{max}, and PV_{max} was observed, nor was there a correlation of MPO_{pre} or MPO_{post} with any of these parameters.

Elevated ΔMPO was associated with increasing number of affected coronaries (1-vessel: 384.2 ± 681.8pM; 2-vessels: 903.2 ± 469.6pM; 3-vessels: 1175.9 ± 785.8pM, $p < 0.01$; Fig. 2). ΔMPO correlated with the number of affected vessels ($r = 0.34$, $p < 0.01$).

A significant increase for MPO_{post} was observed with the increase of affected vessels (1-vessel: 1203[IR:804–1517]pM; 2-vessels: 1471[950–1841]pM; 3-vessels: 1658[IR:1125–2455]pM; $p < 0.05$). In contrast to MPOpre, MPO_{post} correlated with the number of vessels affected ($r = 0.29$; $p < 0.01$). There was no difference in degree of coronary calcification between patients with low and high ΔMPO (χ^2: 2.63, $p = 0.45$).

In vitro administration of heparin showed a dose-dependent release of MPO from collagen (fold increase of MPO activity after 20 min incubation with 0.5, 1.0, 2.5 and 10 U/ml of heparin: 5-fold, 5.3-fold, 6.2-fold and 7.2-fold).

In the 6 separate patients from whom blood samples were collected in the aorta, the coronary sinus and the femoral vein, heparin administration resulted in a more rapid increase of MPO plasma levels in aortic and coronary sinus blood compared to venous blood, indicating a predominant role of MPO deposition in the coronary circulation in CAD patients (Fig. 2). After 5 min, equally elevated MPO plasma levels were observed at all collection sites. No increase in elastase plasma levels was observed, excluding PMN activation.

3. Discussion

In this study we demonstrate that: 1) patients with a high degree of heparin-induced mobilization of vessel-bound MPO exhibit a higher angiographically detectable coronary plaque burden, and 2) heparin-induced mobilization of vessel-bound MPO increases with the number of coronary arteries affected.

MPO has emerged as a powerful predictor of cardiovascular outcome in patients with acute coronary syndromes [16–18], heart failure [19], peripheral artery disease [20] and carotid artery disease [21]. However, conflicting results exist concerning the role of MPO plasma levels in stable CAD [22,23]. A possible confounding factor might be the vascular deposition of MPO.